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Chemical conditions limiting survival and growth of *Galium odoratum* (L.) Scop. in acid forest soil

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Abstract

The performance of *Galium odoratum* in acid forest soil and synthetic soil solutions was studied experimentally. Transplanted *Galium* was unable to grow at a pH (KCl) of 3.3 or lower, and growth was very poor at pH (KCl) 3.5, which corresponds to pH ca. 3.8 in the soil solution. *Galium* was unable to grow at pH 3.5 in experiments using sand perfusion culture with synthetic soil solution with the composition of an acid beech forest soil, whereas some growth was obtained when pH was raised to 4.0. No partial effect of the ambient Al concentration (0.07 mM) of the soil solution was detected, and growth was not influenced by Mn within the range 0.02-0.46 mM. There was little or no difference between plants of five different origins as to their survival and relative growth rate at different soil pH. Establishment from seeds was even more sensitive to soil of a low pH than survival and growth of transplanted mature plants. It is concluded that a high H ion concentration in the soil, at the level characteristic of many Swedish beech forests today, limits survival and growth.

Keywords: *Galium odoratum*, soil, soil solution, pH, aluminium, manganese, survival, growth, south Sweden, beech forest.

Résumé

On a étudié expérimentalement le comportement de *Galium odoratum* dans un sol de forêt acide et dans des solutions artificielles de sol. Transplanté, *Galium* n'arrive pas à croître à un pH (KCl) de 3,3 ou moins, et sa croissance est très faible à un pH (KCl) de 3,5, ce qui correspond à un pH d'environ 3,8 dans la solution de sol. *Galium* n'arrive pas à croître à un pH de 3,5 dans les expérimentations qui utilisent une culture sur sable avec une solution artificielle de sol dont la composition est celle d'un sol acide de hêtraie; mais une faible croissance apparaît lorsque le pH est élevé à 4,0. Aucun effet partiel de la concentration ambiante en Al (0,07 mM) de la solution de sol n'a été détecté et la croissance n'a pas été influencée par Mn entre 0,02 et 0,46 mM. On a trouvé peu ou pas de différences entre des plantes de 5 origines différentes en ce qui concerne la survie et le taux de croissance relatif à différents pH du sol. L'établissement à partir de graines est même plus délicat dans un sol à pH bas que la survie et la croissance de plants matures transplantés. En conclusion, une forte concentration d'ion H dans le sol, au niveau caractéristique de nombreuses hêtraies suédoises aujourd'hui, limite la survie et la croissance.

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INTRODUCTION

Previous studies have demonstrated decreasing populations of *Galium odoratum* over several decades in the deciduous forest of south Sweden, mainly regardless of management of the forest stands (Falkengren-Grerup, 1986; Falkengren-Grerup & Tyler, 1991 *a*). Largescale changes in soil chemical properties have taken place simultaneously, at least partly as a result of the atmospheric deposition of acidifying pollutants. These changes are demonstrable as decreasing soil pH (Falkengren-Grerup, 1986; Hallbäcken & Tamm, 1986), as decreasing pools of exchangeable base cations (Falkengren-Grerup *et al.*, 1987; Falkengren-Grerup & Tyler, 1991 *b*) and as increased solubility of toxic metals, especially Al (Berggren *et al.*, 1990).

Galium odoratum was formerly an important plant in the beech forest vegetation of Scania, south Sweden (Lindquist, 1931). Nowadays it is confined to sites where the pH (KCl) of its rhizosphere soil still exceeds 3.3 and most often 3.6 (Falkengren-Grerup & Tyler, 1991 *a*). Much of the area covered by beech has nowadays a topsoil pH (KCl) lower than 3.3.

It may be hypothesized that the low and decreasing pH of the south Swedish forest soils is a main, direct or indirect, explanation of the decline of *Galium odoratum*. Indirect effects could be the accompanying decreases in the pools of available plant mineral nutrients, or the enhanced soil solution concentrations of Al or Mn. The aims of this study are to demonstrate, using soil and sand culture/solution experiments, to what extent soil acidity as such, Al or Mn concentrations, or mineral nutrient deficiency are factors limiting the survival and performance of *Galium*. Both survival and growth of mature individuals and establishment from seeds are considered.

MATERIALS AND METHODS

Experiments

Greenhouse and outdoor experiments (I-IV, and V, respectively) were conducted between May 1990 and August 1991 (table I). One experiment (IV) was performed in a sand culture, the others in

Table I. — *A brief outline of the quantitative layout of the experiments. Difference between means was tested using ANOVA followed by Tukey test.*

Exp. No.	Subs-trate	Plant material	Treatments	pH (KCl) of soil; range attained
I	Soil	Mature	$CaCO_3$ and/or $MgCO_3$, phosphate, NH_4NO_3, $CaCl_2$, $MgCl_2$, full nutrient; in combinations.	3.0-6.4
II	Soil	Mature	$CaCO_3$ (8 levels), $SrCO_3$	3.0-4.0
III	Soil	Mature (*)	$CaCO_3$ (5 levels)	3.1-4.5
IV	Sand/ solution	Mature	Percolation culture. Synthetic soil solution and 25% Hoagland solution. Al 0.00-0.07 mM, Mn 0.02-0.46 mM.	3.5 and 4.0 in solution
V	Soil	Seeds	$CaCO_3 + MgCO_3$	3.0-4.8

(*) From five sites differing in soil properties.

soil from a beech forest at S:t Olof (55°38'N, 14°07'E) in south Sweden. It was a dystric cambisol with some characters transitional to a podzol, derived from a sandy gneiss-sandstone moraine. The pH (KCl) was low, 3.1, and the pH of the soil solution ca. 3.5. The organic H horizon, a few centimetre deep, was removed and the mineral Ah horizon down to 5 cm, containing 15-18% organic matter was used. The vegetation of the forest floor was very scanty. The soil was sifted (mesh size 6 mm) and thoroughly mixed to form one uniform sample before subsampling for each treatment. Throughout, freshly collected soil was used.

Soil water content in the greenhouse experiments was kept at 50-60% of the water-holding capacity (WHC) by addition every day of synthetic rainwater (pH 4.2; macroconstituents, µmol l^{-1}, being NaCl 73, NH_4NO_3 70, $CaCl_2$ 19, $MgSO_4$ 8, K_2SO_4 7, NaH_2PO_4 3). Temperature in the day was kept at 18-25°C, in the night at 10-16°C. Light conditions and more details on treatments are described in connection with each experiment.

Soil and soil solution analysis

pH (KCl) and pH (H_2O) were measured electrometrically in the supernatant liquid after shaking of 10 g fresh soil with 50 ml 0.2 M KCl solution and distilled water, respectively, for 2 hours. Soil organic matter content was estimated as loss on ignition. Nitrate and NH_4 were determined in filtrates of the 0.2 M KCl extract using flow injection technique, mainly according to RUZIKA and HANSEN (1981).

Soil solutions were obtained by centrifugating soil, usually at 12,000 r.p.m. for 60 minutes, chiefly according to ADAMS *et al.* (1980) and GIESLER and LUNDSTRÖM (1990). pH was determined directly in aliquots of the centrifugate, and K, Mg, Ca, Mn, Fe, Al, and phosphate after filtering (Acrodisc PF, 0.8 µm Prefilter, 0.2 µm Supor) using AAS for the metals and the antimony-molybdenum blue method (OLSEN & SOMMERS, 1982) for phosphate.

DESIGN AND RESULTS OF THE EXPERIMENTS

Experiment I

The aims of experiment I were to test whether (*a*) *Galium* could successfully be transplanted to this highly acidic soil, on which it does not occur in the field, or (*b*) if establishment could be achieved by raising the pH by adding $CaCO_3$ and/or $MgCO_3$, or (*c*) by adding various mineral nutrients without changing the pH. The chemicals (analytical grade) were carefully mixed into the soil as the solid phase or supplied in water solution (table II). Most of the neutralizing effect of the carbonate addition was produced within 3 days, according to measurements on unplanted subsamples.

Plastic pots with bottom drainage, volume 1 dm^3, were supplied with 0.7 l of lightly packed soil. Planting was performed one week after the addition of the solid chemicals. *Galium* was collected in a beech forest at Torup (55°33'N, 13°13'E). Shoots were cut to leave the two lowest leaf whorls, and the rhizome and roots were shortened to ca. 5 cm. Seven pots, each with two plants, of every treatment were randomized in a greenhouse, where only natural day-light, passing through the crown of a large beech, was provided. Irradiation in the day was between 1,500 and 3,500 lx, which is comparable to light conditions prevailing at the beech-forest floor. The experiment lasted for 65 days (28 May to 1 August 1990). At harvest, the lengths and dry weights of shoots produced were determined and soil pH (KCl) was measured.

All plants died within few weeks without producing any shoot growth in the untreated soil, as in all other treatments involving little or no increase in soil pH,

TABLE II. – *Experiment I. Soil pH (KCl) at harvest and corresponding means of shoot weight and maximum length per plant (14 plants treatment^{-1}). Means lacking common letters differ between treatments* ($p < 0.05$).

Treatment	Rate (g dm^{-3})	pH [KCl (**)]	Shoot weight (mg)	Shoot length (mm)	No. of surviving shoots
Control	0	3.0-3.1	0^{d}	0^{d}	0
$CaCO_3$	2.0	4.2-4.3	96^{ab}	88^{a}	12
$CaCO_3$	5.0	5.9-6.0	110^{ab}	98^{a}	13
$MgCO_3$	2.0	4.4-4.5	23^{c}	31^{c}	14
$MgCO_3$	5.0	6.0-6.2	1^{d}	0^{d}	3
$CaCO_3 + MgCO_3$	1.75 + 0.25	4.3	108^{ab}	116^{a}	14
$CaCO_3 + MgCO_3$	4.38 + 0.62	5.9	151^{a}	112^{a}	13
$CaCl_2$ (***)	2.2	3.1	0^{d}	0^{d}	0
$MgCl_2$ (***)	2.0	3.1	0^{d}	0^{d}	0
$CaCl_2 + MgCl_2$ (***)	1.90 + 0.30	3.1	0^{d}	0^{d}	0
$CaHPO_4 + MgHPO_4$	0.47 + 0.055	3.2	0^{d}	0^{d}	0
$CaCO_3 + MgCO_3$ $+ CaHPO_4 + MgHPO_4$	1.75 + 0.25 + 0.47 + 0.055	4.4	84^{b}	86^{b}	13
$CaCO_3 + MgCO_3$ $+ CaHPO_4 + MgHPO_4$	4.38 + 0.62 + 0.47 + 0.055	6.4	99^{ab}	111^{a}	13
NH_4NO_3 (***)	0.20	3.1	0^{d}	0^{d}	0
$CaCO_3 + MgCO_3$ $+ NH_4NO_3$ (***)	1.75 + 0.25 + 0.20	4.2	86^{b}	111^{a}	13
Full nutrient (blåkorn) (*)	0.60	3.2	0^{d}	0^{d}	0

(*) Containing 6% NO_3-N, 6% NH_4-N, 5% P, 14% K, 1.2% Mg, 0.2% Fe, 6% Ca, 0.1% Mn, 0.02% Zn, 5×10^{-4}% Mo, 0.05% B, 8% SO_4-S, <0.4% Cl.

(**) Range of three replicates.

(***) Supplied in water solution.

i.e., addition of $CaCl_2$, $MgCl_2$, phosphate, NH_4NO_3, or even a "full nutrient" (table II). *Galium* was obviously unable to survive in the acid beech forest soil unless pH was raised. Raising the pH (KCl) to 4.3 or 6.0 using $CaCO_3$ only or a mixture of $CaCO_3$ and $MgCO_3$ resulted in vigorous growth with no significant difference between the pH levels, while addition of $MgCO_3$ only caused toxic effects. Inclusion of phosphate or mineral N in the carbonate mixture did not increase growth above that obtained with carbonate(s) only.

Experiment II

The aims of experiment II were to investigate the effects of a more graduated $CaCO_3$ treatment and to elucidate whether similar effects could be obtained by raising the pH using carbonate of the non-essential element Sr without Ca or Mg addition. The chemicals were mixed into the soil one week before planting. *Galium* was collected and treated as in experiment I. 14 replicate pots (28 plants) were run in each of the 9 treatments for a period of 68 days (30 August to 6 November 1990). The pots were arranged in a random design. Additional light was provided using Hg lamps yielding 6,000-7,000 lx between 7 a.m. and 4 p.m. Lengths and dry weights of new shoots were determined and the development of roots + rhizomes was estimated (table III).

TABLE III. – *Experiment II. Soil pH (KCl), extracted amounts of NO_3 and NH_4, and pH of soil solution at harvest. Corresponding means are given for weight of shoot, rhizome length, maximum shoot length and relative root development (classes 0-6; 0 = no growth, 6 = vigorous growth; 28 plants treatment^{-1}). Means lacking common letters differ between treatments ($p<0.05$).*

Treatment	Rate (g dm^{-3})	Soil pH (KCl)	pH of soil solution	NH_4 (µmol g^{-1} dw)	NO_3 (µmol g^{-1} dw)	Shoot weight (mg)	Shoot length (mm)	Rhizome length (mm)	Root class	No. of surviving plants
$CaCO_3$	0	3.0-3.1	3.2	3.7	3.4	0^c	0^d	0^d	0.6^d	2
	0.30	3.1-3.2	3.3-3.4	2.3	4.2	0^c	0^d	0^d	0.6^d	5
	0.60	3.2-3.3	3.4-3.5	1.1	5.0	0^c	$<1^d$	0^d	0.7^d	4
	0.85	3.3-3.4	3.5-3.6	0.8	5.1	3^c	11^c	49^{cd}	1.3^c	16
	1.10	3.4-3.5	3.7-3.8	0.5	4.5	20^b	44^b	67^{bc}	2.5^b	22
	1.40	3.6-3.7	4.0	0.4	3.9	40^{ab}	62^a	86^{ab}	3.8^a	24
	1.60	3.7-3.8	4.1-4.2	0.3	3.4	43^{ab}	74^a	121^a	4.0^a	23
	2.00	3.9-4.0	4.4	0.3	2.7	56^a	71^a	137^a	3.8^a	22
$SrCO_3$	2.70	3.8-3.9	4.3-4.4	0.5	3.9	14^c	27^b	36^d	3.6^a	27

Few plants survived in the soil treated with $CaCO_3$ at the rate of 0.60 g dm^{-3} or less, and growth was very poor also with 0.85 g dm^{-3}, which raised pH (KCl) to 3.3-3.4 and soil solution pH to ca. 3.6. However, growth of rhizomes seemed to be faster than shoot growth at this pH level. Higher rates of $CaCO_3$ improved growth of all biomass parts. Nitrogen mineralization rate probably did not limit growth in any treatment, as slightly elevated concentrations of NO_3 were present in the soil at the end of the experiment, and *Galium* is able to use NO_3 as N source (BOGNER, 1968). The $SrCO_3$ treatment raised the soil solution pH to ca. 4.4. Survival was nearly complete and root development was almost as vigorous as in the comparable $CaCO_3$ treatment, though shoot and rhizome growth was retarded.

Experiment III

Galium in experiments I and II originated from a site with a comparatively high soil pH. The aims of experiment III were to assess the general validity of the findings using *Galium* from five geographically and edaphically differing sites (table IV) planted in the acidic soil used above. Calcium carbonate, used to attain 5 pH levels (table V), was mixed into soil from the same site as in the preceding experiments, one week before planting of *Galium*. Four plants were placed in 21 pots using five replicates (20 plants) and two unplanted pots for each of the five treatments. Natural day-light only was provided, passing through tree crowns and the transparent polycarbonate material of a greenhouse, the intensity being 1,500-4,000 lx during day time. The experiment lasted for 45 days (4 June to 19 July 1991). At harvest, the dry weights of new shoots, rhizomes and roots were measured.

TABLE IV. – *Properties of the beech forest topsoils* (0-5 *cm depth*) *at the five different* Galium *sites. Coordinates of the sites: Anklam (An: 55°37′N, 13°46′E), Kronovall (Kr: 55°39′N, 14°3′E), Maltesholm (Ma: 55°54′N, 13°58′E), Torup (To: 55°33′N, 13°13′E), Fyledalen (Fy: 55°34′N, 13°51′E).*

Sites	An	Kr	Ma	To	Fy
pH (KCl)	3.5-3.6	3.8-3.9	4.3-4.7	4.5-5.3	4.8-5.3
pH (H_2O)	4.3-4.5	4.8	4.9-5.6	5.7-6.0	5.9-6.1
Organic matter, % dry wt.	22.1	18.1	11.8	10.2	18.6
Clay content, % dry wt.	3	10-17	5-8	6-9	25-30

Soil water and soil pH (KCl) was analyzed for one of the plant origins at the end of the experiment.

Regardless of origin, *Galium* proved unable to grow and usually died in the untreated soil (table V). When pH (KCl) was raised to 3.5-3.6, growth of shoots, rhizomes and roots improved considerably, the shoots to about half of the maximum attained at higher treatments. The two highest treatments [pH (KCl) 3.9 and 4.5] resulted in higher shoot and root growth rates, whereas growth of rhizomes did not differ significantly between the three highest treatments. There were little or no differences between plants of the five origins as to their survival and growth at the two lowest treatments. However, biomass production at pH (KCl) > 3.3 was higher in plants originating from the two most acid sites (An, Kr) than in plants from the two least acid sites (To, Fy).

TABLE V. — *Experiment III. Soil pH (KCl) at harvest and corresponding means of shoot, rhizome and root weights per plant (20 plants treatment^{-1}). Means lacking common letters differ between treatments ($p < 0.05$). Explanation of sites in table IV.*

Treatment	Rate (g dm^{-3})	pH (KCl)	Shoot weight (mg)					Rhizome weight (mg)					Root weight (mg)				
			An	Kr	Ma	To	Fy	An	Kr	Ma	To	Fy	An	Kr	Ma	To	Fy
$CaCO_3$	0	3.1	$<1^{c}$	0^{c}	$<1^{c}$	0^{c}	$<1^{c}$	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	1^{c}	0^{c}	1^{c}	0^{c}	1^{c}
	0.85	3.3	7^{c}	9^{c}	9^{c}	4^{c}	3^{c}	8^{b}	8^{b}	6^{b}	3^{b}	6^{b}	2^{c}	3^{c}	5^{c}	3^{c}	4^{c}
	1.40	3.5-3.6	61^{b}	49^{b}	45^{b}	19^{b}	31^{b}	25^{a}	18^{a}	14^{a}	14^{a}	13^{a}	23^{b}	22^{b}	17^{b}	12^{b}	18^{b}
	2.00	3.9	73^{b}	100^{a}	81^{a}	42^{a}	54^{a}	26^{a}	18^{a}	18^{a}	13^{a}	13^{a}	29^{ab}	37^{a}	31^{a}	21^{a}	27^{a}
	3.20	4.5	115^{a}	124^{a}	73^{a}	51^{a}	43^{ab}	37^{a}	18^{a}	19^{a}	14^{a}	12^{a}	35^{a}	33^{a}	19^{b}	22^{a}	22^{ab}

The degree of VA mycorrhizal infection was estimated in 20 root samples from each site using KOH pretreatment and staining technique according to PHILLIPS and HAYMAN (1970). In four out of the five origins infection was very low, whereas the presence of strong VA mycorrhiza was observed in most roots from the Ma site, which was intermediate in soil pH. As growth of *Galium* from this site was also intermediate, growth performance did not seem related to the rate of VA infection in any simple way.

TABLE VI. — *Experiment III. pH (KCl) and chemical properties of centrifugated soil solution at harvest, measured in pots planted with* Galium *of the Torup origin and in unplanted soil. P (HAc) measurements ranged between* 0.4 *and* 0.6 *µmol g^{-1} dry wt. in the untreated soil to* 0.3-0.4 *µmol g^{-1} dry wt. in the highest treatment.*

Treatment	Rate (g dm^{-3})	Soil pH (KCl)	Soil solution							
			pH	Ca (mM)	Mg (mM)	K (mM)	Al (mM)	Mn (mM)	Fe (µM)	P (µM)
					Planted					
$CaCO_3$	0	3.1	3.2	0.7-0.8	0.4-0.5	0.4-0.5	0.09-0.11	0.11-0.13	11-13	9-15
	0.85	3.3	3.4	3.1-3.5	0.5-0.6	0.3-0.4	0.06-0.07	0.14-0.16	10	10-15
	1.40	3.5-3.6	3.7	3.4-4.1	0.4-0.5	0.2	0.04	0.10-0.12	8-9	8-10
	2.00	3.9	4.1	3.1-3.8	0.3	0.1	0.03	0.06-0.08	9-11	4-8
	3.20	4.5	4.6	3.8-4.0	0.2	0.1	0.02	0.01	7-8	2-3
					Unplanted					
$CaCO_3$	0	3.1	3.2-3.3	0.7-0.8	0.4	0.4	0.10-0.11	0.11-0.12	12-13	14-15
	0.85	3.3	3.8	3.2-3.6	0.5-0.6	0.3	0.07	0.14-0.16	9	10
	1.40	3.6	4.2	4.1-4.5	0.5	0.3	0.04-0.05	0.13-0.14	8	9-10
	2.00	3.9	4.2	4.2-4.3	0.4	0.2	0.03	0.09	8-9	6-7
	3.20	4.5	4.8	4.4-4.9	0.3	0.1	0.02	0.01	6-7	2-3

pH of the soil solution increased gradually with increasing $CaCO_3$ addition rates (table VI). There was a sudden rise in the concentration of Ca with the lowest $CaCO_3$ treatment, but no further increase in Ca was measured at higher $CaCO_3$ additions. Concentrations of other elements analyzed (Mg, K, Mn, Fe, Al, and P), however, were inversely related to the $CaCO_3$ addition. As the decrease was almost similar in planted and in unplanted pots, mineral nutrient uptake by the plants was not the main reason for the decrease. Chemical precipitation is the most probable explanation of the decrease in Al and Mn concentrations. The decreasing soil solution concentrations of Al and/or Mn might have contributed to the positive effect of $CaCO_3$. It seems less probable that deficiency of K, Mg, Mn, Fe, or P limited the survival and growth in this soil, considering the results of experiment I and the fact that the highest concentrations were measured in the solution of the untreated soil. From the same comparison it seems less likely that deficiency of Ca in the untreated soil was a reason for the failure of *Galium*, as the lowest $CaCO_3$ addition raised the Ca concentration of the soil solution to almost the same level as in the highest treatments, without improving growth significantly.

Experiment IV

The aims of experiment IV were to test the sensitivity of *Galium* to high acidity, Al and elevated Mn concentrations using well-defined synthetic nutrient and soil solutions in sand percolation culture. A pure quartz sand, mean particle size 0.65 mm, was washed in 0.1 M HCl for 24 hours followed by several washings in distilled water. Plastic pots with bottom drainage containing 0.5 l of sand were perfused, before planting, with the treatment solutions. These were one synthetic soil solution with the same inorganic ion composition as the solution of the soil used in experiments I-III, and a 25% Hoagland solution as a comparison. All treatments included pH 3.5, being the pH of the soil solution, and pH 4.0 (table VII). The synthetic soil solution was used at zero and at ambient concentration of Al and at ambient and elevated concentrations of Mn (table VIII).

Galium was collected at the Torup site in early spring, when the shoots were only a few centimetres tall. Shoots were left untouched, rhizomes + roots were cut to ca. 4 cm length. 7 pot replicates (14 plants) were used in each treatment. The 0.5 l sand volume in each vessel was perfused every second day by 200 ml solution, allowed to drain freely through the system. Artificial light was provided during $12\,h\,d^{-1}$ using 400 W Philips Son-T Agro lamps which yielded 7,000-9,000 lx at the surface of the pots. The experiment lasted 42 days (20 February to 2 April 1991). At harvest, shoot lengths were measured and root development was estimated (table VIII).

Plants exposed to the synthetic soil solution at ambient pH (3.5) almost failed to grow and most plants died during the course of the experiment. Growth in the 25% Hoagland solution at pH 3.5, and in the soil solution at pH 4.0 was moderate. Exclusion of Al (0.07 mM) or increasing the Mn concentrations from 0.02 over 0.18 to 0.46 mM in the synthetic soil solution did not influence growth in a measurable way, though survival was better and there was a tendency of better root development without Al. The best growth, in particular of the below-ground parts, was obtained with 25% Hoagland at pH 4.0.

Successful reestablishment and growth could thus not occur when the chemical composition and pH of the solution was close to that of the field soil used in the preceding experiments. This will make any consideration of potentially toxic organic compounds unnecessary in explaining the failure of *Galium* in this soil. Increasing the pH to 4.0 improved conditions to some extent and improved them greatly,

TABLE VII. – *Experiment IV. Chemical composition of the nutrient solutions as compared to soil solution from percolation lysimeters in the rhizosphere of* Galium *in* 18 *beech sites. The synthetic solution simulates the soil solution of the experimental soil in February* 1991, *centrifugated at* 5,000 r.p.m., 1 *hour. The Hoagland solution used (SIGMA H*2395) *was diluted to* 25%, *pH was adjusted to* 3.5 *or* 4.0 *by adding HCl.*

	Synthetic soil solution	25% Hoagland solution	Soil solutions from *Galium* sites mean (range)
pH	3.5; 4.0	3.5; 4.0	5.4 (3.8-6.2)
Concentrations, mM:			
Na	0.49	0	1.1 (0.5-2.2)
K	0.20	1.51	0.2 (0.1-0.3)
Mg	0.08	0.49	0.2 (0.1-0.4)
Ca	0.19	0.70	0.5 (0.2-1.1)
Mn	0.022	0.002	0.03 (0.01-0.07)
Fe	0.005	0.005	0.02 (0.01-0.05)
Zn	2.10×10^{-3}	0.18×10^{-3}	$1.12\ (0.15\text{-}3.9) \times 10^{-3}$
Cu	0.22×10^{-3}	0.08×10^{-3}	–
B	0.005	0.012	–
Mo	0.07×10^{-3}	0.11×10^{-3}	–
Al	0.07	0	0.05 (0.03-0.11)
NH_4	0.20	0.25	–
NO_3	0.59	2.90	–
SO_4	0.10	0.50	–
Cl	0.63	<0.01	–
P	0.04	0.25	–

TABLE VIII. – *Experiment IV. Means of shoot weight and relative root development per plant (classes* 0-6) *at harvest* (14 *plants treatment*$^{-1}$). *Root development estimated as in table III. Means lacking common letters differ between treatments* ($p < 0.05$).

Treatment	pH	Shoot length (mm)	Root class	No. of surviving plants
Soil solution	3.5	4^{c}	$<0.1^{c}$	2
Soil solution	4.0	24^{b}	0.3^{bc}	9
Soil solution, −Al	3.5	9^{c}	0.1^{bc}	5
Soil solution, −Al	4.0	28^{b}	0.6^{b}	11
Soil solution, 0.18 mM Mn	3.5	5^{c}	$<0.1^{c}$	2
Soil solution, 0.18 mM Mn	4.0	28^{b}	$<0.1^{c}$	9
Soil solution, 0.46 mM Mn	3.5	4^{c}	0^{c}	3
Soil solution, 0.46 mM Mn	4.0	28^{b}	0.3^{bc}	9
25% Hoagland	3.5	26^{b}	0.4^{b}	10
25% Hoagland	4.0	60^{a}	2.4^{a}	13

when the concentrations of most nutrients were higher, as in the Hoagland solution. The Al concentration of the soil solution was not distinctly, and Mn concentrations not at all growth-limiting.

The soil solution chemistry of our experimental soil was related to rhizosphere soil water concentrations in 18 beech forest sites, where *Galium odoratum* actually occurs (table VII). Concentrations of several nutrients, including Mg, Ca, Zn, and Fe, were higher than in the experimental soil solution. Mean pH was clearly higher (5.4), whereas concentrations of K, Mn, and Al were similar.

Experiment V

The aims of experiment V were to elucidate whether germination and growth in soil adjusted to different pH using carbonates were similar to the reestablishment of adult plant material. Soil of the same origin and properties as in the previous experiments was used and $CaCO_3 + MgCO_3$ (4 : 1; 0.60-3.60 g dm^{-3} of soil) was added one week before sowing to attain 9 pH levels. In each treatment seeds of *Galium*, originating from the Torup (To) site, were evenly distributed in two vessels, volume 0.7 l, surface area 0.7 dm^2; 100 seeds per vessel. Seeds of *Galium* usually do not germinate in short-term experiments and probably need to hibernate in soil. Therefore, the vessels were randomized within a small area in a beech forest, exposed to ambient weather conditions but protected against vertebrate disturbance by a fence. The experiment lasted 235 days (from 31 October 1990 to 22 June 1991).

Few plants survived in the soil treated with carbonates at the rate of 1.40 g dm^{-3}, pH (KCl) 3.7, or less (fig. 1). A sudden improvement of survival and growth took place at 1.60 g dm^{-3} [pH (KCl) 3.8], and a further improvement of both shoot and rhizome + root growth occurred between 2.00 and 2.60 g dm^{-3} [pH (KCl) 4.0 and 4.3]. The highest rate, 3.60 g dm^{-3} [pH (KCl) 4.7-4.8], did not seem to increase growth any further. Above and below ground growth was related to treatment rates in a similar manner. Plant establishment from seeds and growth of seedlings were thus more sensitive to low soil pH than survival and growth of adult plants.

DISCUSSION

The distribution of *Galium odoratum* in south Sweden is favoured by forest soils with a relatively high pH. Due to the acidification of soils, *Galium* nowadays occurs at a somewhat lower pH than in the 1920's and, above all, it has decreased markedly in abundance (Falkengren-Grerup, 1990). In Poland, *Galium* is found at a topsoil pH (KCl) of 3.3-6.3, though it is most abundant in the middle of this range (Czarnecka, 1986). Analyses in Britain and Central Europe over the last few decades have not shown any similar decrease and possible frequency changes might have been concealed by large changes in forest management (Tregenza, 1986; Wilmanns & Bogenrieder, 1986; Kuhn *et al.*; 1987; Wilmanns, 1989).

The soil experiments in our study demonstrated that appreciable survival and growth of transplanted plants demanded a pH (KCl) of at least 3.5. These results are in good agreement with observations from the field. Differences among *Galium* originating from five edaphically and geographically differing sites were very small

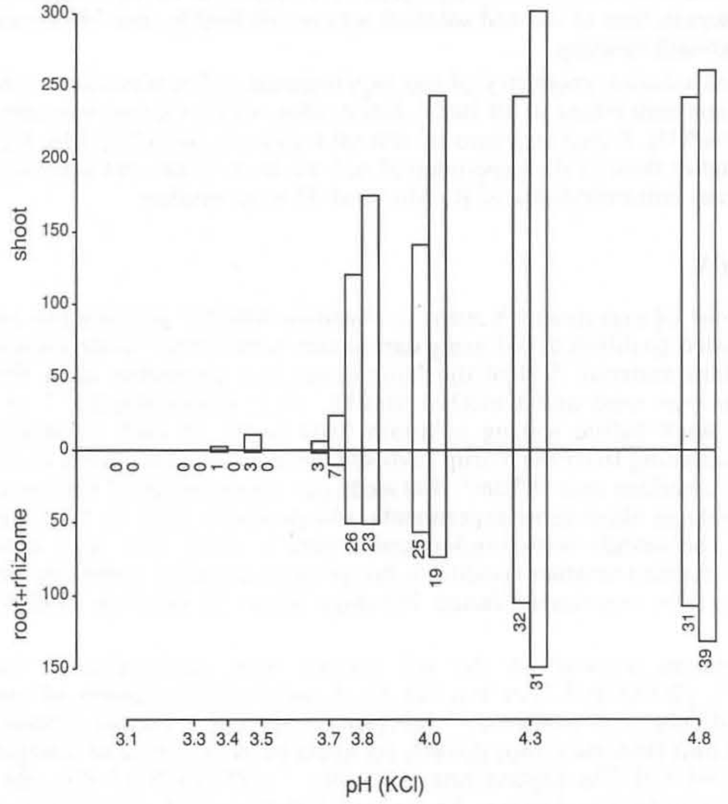

FIG. 1. — Experiment V. Total weight per pot of shoot and root + rhizome biomass. Number of plants produced (from 100 seeds) are given for the nine pH (KCl) levels attained.

regarding their relative growth response to soil pH, though differences were observed in the absolute growth rates.

The establishment from seeds seems to be even more susceptible at a low pH, as a reasonable growth was only measured from about pH (KCl) 3.8. These results could offer an additional explanation of the frequency changes in recent decades. The observed decreases in cover could be due to absence of sexual reproduction, whereas vegetative growth of old, established individuals at a lower pH could explain the fact that some *Galium* still remains on quite acid soils. By adding nutrients to the acid soil or by raising soil pH, using different chemicals, it was concluded that growth related best to the hydrogen ion concentration and not to nutrient deficiency of the acid soil. However, in raising soil pH, several other conditions also change. Chemical gradients of the soil solution related to treatments were created (table VI), which could have affected the plants. In particular the concentrations of Al and Mn decreased as a result of the treatments. A possible toxicity of Al and Mn at ambient and elevated concentrations of macro nutrients were therefore tested in sand percolation culture.

The Al concentration of the soil solution, in the synthetic solution mainly present in monomeric Al^{3+} form, did not influence growth, nor did a 20 times elevated concentration of Mn^{2+}. Survival and growth were much higher at pH 4.0 than at pH 3.5. This interval corresponds well to the pH interval in the soil experiment, where survival and growth changed from very poor or none to good. Shoot growth was generally higher in the Hoagland solution than in the synthetic soil solution, markedly higher at pH 4.0. Better growth at a high soil solution pH may arise from synergistic or antagonistic interactions between ions, e. g., manifested as a higher tolerance to a low soil pH at high Ca concentrations, or a pH increase in the root environment at a high $NO_3:NH_4$ ratio (RUNGE & RODE, 1991). Hydrogen ion effects may arise from disturbance of the plasmalemma function and irreversible damage may occur at high H^+ concentrations (FAWZY *et al.*, 1954). It is important that solutions containing field-relevant levels and proportions of elements are used in studies aiming at elucidating soil factors of importance to the survival and performance of plants.

The centrifugation technique is a convenient method to obtain soil solution for analysis without appreciably changing its chemical state. Synthetic soil solutions of a field relevant composition may thus be designed, as in our study. However, variations certainly occur between sampling occasions, particularly in the concentrations of essential nutrients, which may be higher in the winter time when uptake by vegetation is at minimum (ZABOWSKI & UGOLINI, 1990). The synthetic soil solution used was copied from the acid forest soil used in four out of the five experiments. By comparing soil solutions from a number of *Galium* sites distributed along the soil pH gradient with that of our experimental soil, it became evident that the largest difference was pH. Calcium, Mg, and Fe were close to the minimum of the *Galium* sites, though still within the range of tolerance of this species, while K, Al, Zn, and Mn were similar to the average.

CONCLUSIONS

1. Throughout, transplanted *Galium* required a pH (KCl) > 3.3 to grow, and growth was very poor at pH (KCl) 3.5, which is comparable to the lower limit of its occurrence in the field.

2. Addition of nutrients, single or in combinations, did not improve survival and growth unless pH was raised.

3. pH (KCl) 3.5 corresponds to a pH of ca. 3.8 in the soil solution. Survival and growth in a synthetic soil solution of pH 3.5 (comparable to the solution of the acid forest soil studied) were very poor, whereas some growth was obtained when pH was raised to 4.0. No partial effect of the ambient Al concentration (0.07 mM) was detected, and growth was not influenced by Mn within the range 0.02-0.46 mM.

4. Plants of five different origins had similar survival and relative growth in relation to soil pH.

5. Establishment from seeds proved to be even more sensitive to soil with a low pH than survival and growth of transplanted plants.

6. It is concluded that a high H^+ concentration in the soil limits survival and growth of *Galium odoratum* in forest soil subjected to acid rain and decreasing pH.

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